Effects of GF-120 Fruit Fly Bait Concentrations on Attraction, Feeding, Mortality, and Control of *Rhagoletis indifferens* (Diptera: Tephritidae)

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ABSTRACT Effects of different concentrations of GF-120 NF Naturalyte Fruit Fly Bait on attraction and feeding responses, mortality, and control of the western cherry fruit fly, *Rhagoletis indifferens* Curran, were determined. In the laboratory, flies that had been exposed to sugar and yeast extract and then deprived of all food for 16–20 h were attracted to 40.0% GF-120, but not to 0.6 and 4.8% GF-120 (vol:vol). Nonstarved flies were not attracted to any concentration. Flies in the field were not attracted to 55.6% GF-120 on cherry leaves, and few flies fed on the bait. In the laboratory, males fed for shorter durations on and ingested lower amounts of 0.6% than 4.8 or 40.0% GF-120, but females fed equally on all concentrations. Spinosad in GF-120 was highly toxic to flies. Lethal concentrations$_{50}$ (LC_{50} values) of spinosad for starved flies at 1–4 d were 1.5–0.7 ppm. When gravid flies were exposed to cherries treated with 0.6, 4.8, and 40.0% GF-120, mortality was greater at each higher concentration, but none prevented oviposition. Field spray tests comparing 0.6, 4.8, and 40.0% GF-120 in 225 ml of spray per cherry tree resulted in 79–94% lower larval infestations than in controls, but no differences were seen among the concentrations. Evidence from this study indicates that fresh 40.0% GF-120 was attractive in the laboratory but that flies were not attracted to fresh GF-120 from far distances within trees, suggesting that suppression of populations is caused in large part by flies finding the bait through normal movement over large areas.

KEY WORDS *Rhagoletis indifferens*, GF-120 NF Naturalyte Fruit Fly Bait, spinosad concentrations, attraction, feeding

WESTERN CHERRY FRUIT FLY, *Rhagoletis indifferens* Curran, has been the major insect pest of sweet cherries, *Prunus avium* (L.) L., in the Pacific Northwest of the United States since at least the 1940s (Frick et al. 1954). In cherry-growing areas, *R. indifferens* occurs mostly in unmanaged residential trees, which are probably the main sources of infestations in commercial orchards. Insecticide sprays in orchards need to eliminate larval infestations to be considered effective because of the zero tolerance for larvae in exported fruit. Organophosphate and carbamate insecticides (Zwick et al. 1970, 1975) have been successfully used in the past to meet the zero tolerance and are still available for use today. However, because of anticipated restrictions on their use in the near future due to the Food Quality and Protection Act of 1996, the evaluation of more environmentally acceptable alternatives is increasingly important. One such alternative is GF-120 NF Naturalyte Fruit Fly Bait (Dow AgroSciences, Indianapolis, IN), an organically labeled product that contains spinosad, an insecticide derived from fermentation products of the soil bacterium *Saccharopolyspora spinosa* Mertz & Yao, and a mix of sugar, protein, ammonium acetate, and other ingredients. Spinosad, GF-120, or GF-120 bait alone (without spinosad) are effective in killing, managing, or attracting several tropical and subtropical tephritids (Adán et al. 1996, King and Hennessey 1996, Burns et al. 2001, Vargas et al. 2002, Barry et al. 2003, Fabre et al. 2003, Prokopy et al. 2003, Revis et al. 2004).

GF-120 was developed for control of exotic fruit flies based on feeding responses of Mexican fruit fly, *Anastrepha ludens* (Loew); West Indian fruit fly, *Anastrepha obliqua* (Macquart); and Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Moreno and Mangan 2003), but preliminary tests in 2003 in Washington state suggested GF-120 sprays also are effective in controlling *R. indifferens* (Hansen 2004). How GF-120 controls *R. indifferens* and the effects of factors such as bait concentration and fly hunger state on feeding responses to and mortality caused by GF-120 have not been studied. For other *Rhagoletis* species, various protein baits are only moderately attractive at best (Neilson 1960, Reissig 1977, Hendrichs et al. 1990, Barry and Polavarapu 2004). A recent study also showed that blueberry maggot, *Rhagoletis mendax* Curran, is not attracted to GF-120 (Pelz et al. 2005). Thus, a strong attraction to the volatiles in these baits may not be a major mechanism of controlling temperate fruit flies. High bait concentrations should result in greater fly attraction, higher feeding responses, and higher mortality than low concentrations. Nutri-

tional state also should affect attraction, feeding, and mortality. A positive relationship between protein-deprivation and attraction to protein baits has been shown consistently in fruit flies (Hendrichs et al. 1990; Robacker 1991; Prokopy et al. 1992, 1993; Vargas et al. 2002).

In this study, the major objectives were to determine the effects of GF-120 concentrations on the attraction, feeding, and mortality responses of *R. indifferens.* A supporting objective was to determine the effect of hunger state on these responses. In addition, GF-120 concentrations studied in the laboratory were tested for control of *R. indifferens* in the field. A goal was to form a hypothesis for the mechanism of control by GF-120 bait sprays based on behavioral and field efficacy data.

Materials and Methods

Fly Sources and GF-120 NF Naturalyte Fruit Fly Bait. Flies used in laboratory experiments originated as larvae in infested cherries collected in June and July 2003 and 2004 in central Washington. Pupae were maintained in moist soil at 3°C for 6 to 7 mo before being transferred to 27°C for adult emergence. Before tests, flies were maintained in 30 by 30 by 30-cm screen cages with water and a dry 20% yeast extract (EZ Mix, Sigma, St. Louis, MO) and 80% sucrose (wt:wt) mix on paper strips. The term "food" in experiments below refers to this mix. The undiluted GF-120 NF Naturalyte Fly Bait (density 1.2 g/ml) used in experiments contained 0.02% spinosad (wt:vol, or 0.24 g/liter) and was a brown liquid concentrate with an odor similar to vinegar and with a pH of 4.7. Total solids were 57.5% (wt:wt). High-performance liquid chromatography analysis using a refractive index detector (Agilent 1100 Series RID, Agilent Technologies, Palo Alto, CA) indicated 29.7% of the solids were sugars (9.6% fructose, 6.9% glucose, and 13.2% sucrose). Use of standard method (SM) 4500 for total Kjedahl nitrogen indicated undiluted GF-120 was 12.3% protein, and use of this method for NH_3-H (Clesceri et al. 1998) indicated it was 0.56% ammonia (wt:wt). GF-120 has the same ingredients as its precursor, Solbait (USDA–ARS, Weslaco, TX) (Moreno and Mangan 2003), except it contains propylene glycol instead of polyethylene glycol (a synergist), and it is more concentrated than Solbait. Solbait and GF-120 contain Solulys corn protein (Roquette Freres, Lestrem, France), ammonium acetate, polyethylene glycol 200, polysorbate 60 (Tween, a synergist), soybean oil, invert sugar, xanthum gum (starch thickener), and water.

Experiment 1: Attraction of Flies to GF-120 Concentrations in Cages. Tests for this experiment were conducted in a 61-cm-high by 55-cm-wide by 53.5-cm-long plywood cage with organdy screening. The cage was placed beneath two 34-W incandescent light tubes (light intensity of 2–5 W/m^2 within the cage) at 27°C and 30–40% RH. Flies were 3–7 d old when tested. Starved flies had been deprived of food for 16–20 h before tests (same for all tests below). Nonstarved flies were exposed to food up to the time of tests. There was no food during testing. Fresh GF-120 concentrations tested were 0.6, 4.8, and 40.0% (vol:vol, as in all tests), corresponding to 0.004, 0.03, and 0.25% ammonia (from original concentrate, not added), respectively. The 40.0% concentration of GF-120 was that achieved when the concentrate was diluted as per label directions. Ammonia is an attractant for *R. indifferens* (Frick 1952). Water was used for the control. For each test, an artificial magnolia plant (Silk Gardens Shop, Irving, TX) consisting of four silk leaves (13–15 cm in length by 7 to 8 cm in width) attached to a 49.5 cm-tall stem was inserted into an empty 1000-ml Erlenmeyer flask to keep it upright inside the test cage. One treated and one untreated plant were placed 15 cm apart. For the control, both plants were untreated. Water was provided in cotton plugged in the opening of another flask. A 1000-μl volume of GF-120 solution was applied to the upper surface of the four leaves of the treatment plant by using a micropipette, with 250 μl applied on each leaf (as five 50-μl droplets). An equal volume of water was applied on the untreated plant. All leaves were oriented as close to horizontal as possible. Testing was done with bait immediately after application. For each test, 40 starved or nonstarved flies (20 males and 20 females) were released from a 473-ml paper container placed on the bottom of the cage equidistant from the plants. The first observations were made 5 min after release. Numbers of flies landing on the tops and bottoms of leaves at 2-min intervals over 1 h were recorded. Numbers of flies feeding also were recorded. There were five replicates of each treatment.

To determine the attraction of flies deprived of protein from emergence to fresh 40.0% GF-120, two tests essentially identical to the previous ones were conducted, with the only difference being the feeding history of the flies. In test 1, 28–32 5- to 6-d-old flies (equal numbers of males and females) exposed from emergence to only 20% sucrose on a dental wick and to water were released inside a cage. Flies were not starved. There were three replicates. In test 2, because of the possibility that flies exposed to 20% sucrose immediately before testing were too engorged to respond, 16–30 6- to 7-d-old flies (equal numbers of males and females) exposed to 20% sucrose for the first 3 d and 5% sucrose during the last 3 or 4 d up to the test were released inside a cage. There were four replicates of this test.

Experiment 2: Attraction of Flies to GF-120 in the Field. Two field tests were conducted in June and July 2004 to compare the attraction of flies to GF-120 and a known attractant, ammonium hydroxide (AH), which contains 29.3% ammonia. A fresh 55.6% GF-120 concentration (vol:vol), containing 0.31% ammonia (from original concentrate, not added), was compared with an AH lure. This higher than label rate concentration of GF-120 was tested to increase chances flies would be drawn to the bait. The AH lure contained 10 ml of ammonium hydroxide in a Nalgene bottle and released ammonia at a rate of 2 mg/h (Yee and Landolt 2004). GF-120 was applied on five large sweet cherry leaves close together on a 15–20-cm length of branch.

The fresh AH lure was hung from a separate branch. The control consisted of another branch tagged with a small piece of white tape. Treatments were 1 m apart within the same tree on the south side, 1.5–1.7 m above ground. Test one was conducted on three cherry trees by using 2,500 μl of GF-120 solution (500 μl per leaf as five 100-μl droplets) on five dates from 20 May to 24 June in a backyard in Zillah, WA. Test two was conducted on four or six cherry trees by using 5,000 μl of solution (1000 μl per leaf as ten 100-μl droplets) on four dates from 1 to 26 July in rural sites in Roslyn, WA. On 15 June, an additional test was conducted on three trees in the backyard in Zillah without the AH lure to determine whether ammonia interfered with fly attraction to GF-120. In all tests, numbers of flies seen within 30 cm of GF-120-treated leaves, the AH lure, or the tape on the control branch every 2 min over 30 min were recorded. In Zillah, two 30-min observations (averaged for analysis) were made for each tree. Treated leaves were removed and branches were shaken to dislodge flies after the first observations. Treatment positions were then reassigned to different branches. In Roslyn, one 30-min observation was made for each tree. All observations were made between 0900 and 1500 hours. Each tree was considered a replicate.

Experiment 3: Feeding Responses of Starved Flies to GF-120 Concentrations in Vials. In this experiment, individual 4–6-d-old starved flies were presented with 0 (control), 0.6, 4.8, or 40.0% GF-120 inside 5.0 by 1.4-cm glass vials. GF-120 concentrations corresponded to 0.2, 1.4, and 11.9% sugar (wt:wt); pH 6.4, 6.2, and 5.9; and 1, 11, and 89 ppm spinosad, respectively. A 50-μl drop of water or GF-120 solution was placed inside a vial at 21°C. A fly was weighed inside a 1-g gelatin capsule (Eli Lily & Co., Indianapolis, IN) by using a microbalance (Sartorius, Goettingen, Germany) and then placed inside the vial with the drop of GF-120 or water. The fly walked until it encountered the drop. If feeding or drinking did not occur within 15 min, the fly was recorded as a nonfeeder. Flies that fed were removed and weighed again inside the capsule. Afterwards, an individual fly was placed in a 473-ml plastic container with food and water and held at 27°C and 30–40% RH. To determine mortality, survival of feeders and nonfeeders was recorded daily for 14 d. Mortality of some nonfeeders that made contact (feet only or feet and mouthparts) with the bait also was recorded. There were 11–34 flies of each sex tested at each concentration.

Experiment 4: Mortality of Starved and Nonstarved Flies Exposed to GF-120 Concentrations in Cages. To further determine the effects of starvation and GF-120 concentrations on attraction and mortality, 3–7-d-old starved and nonstarved flies were exposed to water or 10 GF-120 concentrations, each on a plastic dish (7 mm in height, 5 cm in diameter) for 1 h. Concentrations were 0 (control), 0.6, 1.2, 2.4, 4.8, 9.1, 16.7, 20.0, 25.0, 33.3, and 40.0%. Starved and nonstarved flies were compared simultaneously. Twenty flies (10 males and 10 females) were removed from 30 by 30 by 30-cm screen holding cages and placed inside a 473-ml paper container with water but no food. A dish containing 100 μl of water or one GF-120 concentration was then introduced onto the bottom of the container. Solutions were presented as five 20-μl drops placed uniformly on the dish. Flies were exposed to solutions for 1 h only to limit incidental contact between flies and solution and to avoid large changes in water concentrations. After 1 h, the bait was removed, and food was introduced into the container. Mortality of flies was recorded at 1, 2, 3, and 4 d after exposure. There were 12 and 10 replicates of the control (starved and nonstarved, respectively) and five replicates of all GF-120 concentration treatments.

Experiment 5: Mortality and Oviposition of Flies Exposed to GF-120 Concentrations on Cherries in Cages. Two tests were conducted to determine mortality and oviposition levels of nonstarved, reproductively mature flies that were continuously exposed to GF-120-treated cherries. Flies ≥14 d old were exposed to sweet cherries treated with water (control) and 0.6, 4.8, and 40.0% GF-120. Cherries ('Bing') were dipped in water or GF-120 solutions for ≈2 s. Three cherries were placed on a plastic dish (5 cm in diameter) on the bottom of a 473-ml paper container. Food and water were provided. Ten flies (five males and five females) were introduced into a container. In test 1, all three cherries were left in the container for 2 d. Fly mortality was recorded each day. There were seven replicates of the control and treatments. After 2 d, cherries from three of the seven replicates were removed and stored in alcohol for later dissection to determine effects of GF-120 on oviposition, whereas cherries from the remaining four replicates were held at 27°C for 4 wk to determine effects on larval emergence. In test 2, all three cherries were left in the container for 4 d. Mortality was recorded at 1, 2, 3, and 4 d after exposure. The numbers of eggs in the three cherries were recorded. There were three or four replicates of the control and each treatment.

Experiment 6: Field Spray Tests of GF-120 Concentrations. Two spray tests were conducted in May to July 2004 in central Washington, each comparing a control and 0.6, 4.8, and 40.0% GF-120. One test was conducted in Yakima and the other in Moxee (≈25 km away). In Yakima, single, isolated sweet cherry trees (3–6 m in height) in residential yards were used, and in Moxee, single sweet cherry trees (3 to 4 m in height) in an experimental orchard were used. A 14 by 23-cm sticky yellow panel trap (Trécé, Salinas, CA) baited with a 10-g ammonium carbonate lure was hung on each tree on 11 May and left on the tree throughout the spray periods. A spray volume of 225 ml was uniformly applied on each tree using a squirt bottle. The initial application was made within 5 d of the first fly catch, with subsequent applications made every 6 to 10 d (rains prevented sprays from being made at regular 7-d intervals). There were five applications in Yakima (17 May, 24 May, 1 June, 7 June, and 14 June) and four in Moxee (4, 14, 22, and 29 June). In Yakima, there were three or four usable treatment and nine control trees scattered at various sites. At Moxee, there were four trees of each treatment and the control,

arranged in a randomized block design with single untreated buffer trees surrounding experimental trees. In Yakima, 200 cherries were picked from each tree on 21 June, and in Moxee, 231–328 cherries were picked from each tree on 1 July. Cherries were laid on hardware cloth in tubs with soil and held outdoors. Numbers of larvae or pupae seen in the soil over 4 wk were recorded.

Statistics. One-way or factorial analysis of variance (ANOVA) was conducted for data in experiments 1–3, 5, and 6, followed by Fisher's least significant difference (LSD) test for mean separation (SAS Institute 2001). Probit analysis (Environmental Protection Agency 2004) was conducted to determine lethal concentrations (LCs) in experiment 4, adjusting for control mortality (Abbott 1925), and analyzing data for each day separately. In experiments 1 and 4, two-way ANOVA also was conducted, with fly starvation category and GF-120 concentration as the two factors. Percentages were square-root arcsine-transformed before analyses. Counts were subjected to square-root $(y + 1)$ transformation. Means ± SE are reported.

Results

Experiment 1: Attraction of Flies to GF-120 Concentrations in Cages. Starved flies were not attracted to 0.6% and 4.8% GF-120, but they were slightly attracted to 40.0% GF-120 ($F = 3.87$; df = 3, 16; $P = 0.0295$) (Table 1). More flies visited the top of leaves with the 40.0% GF-120 than with other concentrations ($F = 7.60$; df = 3, 16; $P = 0.0022$), but similar numbers of flies visited the bottom of leaves across all concentrations ($P > 0.05$) (Table 1), suggesting the bait attracted flies to the leaf surfaces. Flies fed significantly only on 40.0% GF-120 ($F = 11.16$; df = 3, 16; $P = 0.0003$). Nonstarved flies did not respond to any of the concentrations ($P > 0.05$) (Table 1). Two-way ANOVA indicated that more starved than nonstarved flies were attracted to the GF-120 and that 40.0% GF-120 was most attractive (starvation category: $F = 29.25$; df = 1, 32; $P < 0.0001$; concentration: $F = 4.43$; df = 3, 32; $P = 0.0103$; interaction: $F = 1.01$; df = 3, 32; $P = 0.4032$). Starved flies were more attracted to treated than untreated leaves (0.6%: $F = 4.90$, $P = 0.0578$; 4.8%: $F = 12.60$, $P = 0.0075$; 40.0%: $F = 14.02$, $P = 0.0057$; df = 1, 8), whereas nonstarved flies landed on both equally ($P > 0.05$).

Five- to 6-d-old flies deprived of protein from emergence but with access to 20% sucrose up to testing were not attracted to 40.0% GF-120, because mean percentages of flies landing on treated and untreated leaves were 0.23 ± 0.12 and 0.03 ± 0.03%, respectively ($P > 0.05$). Six- to 7-d-old flies exposed to 20% sucrose for the first 3 d and 5% sucrose during the last 3 or 4 d up to testing also were not attracted, because mean percentages landing on treated and untreated leaves were 0.00 ± 0.00 and 0.38 ± 0.38%, respectively ($P > 0.05$).

Experiment 2: Attraction of Flies to GF-120 in the Field. In test 1 at the Zillah site (Table 2), there was no difference between numbers of flies seen near the 2,500 μl of 55.6% GF-120 and the control over 30-min observations, but on three of 5 d, numbers near the AH lure were greater than near the GF-120 treatment and control ($F = 9.80–32.72$; df = 2, 6: $P = 0.0129–0.0006$) (Table 2). In test 2 at the Roslyn site (Table 2), there was also no difference between fly numbers near the 5,000 μl of GF-120 and the control, but on two of 4 d, more flies were near the AH lure than near the GF-120 treatment or control ($F = 3.61$ and 30.33; df = 2, 15 and 2, 12; $P = 0.0524$ and <0.0001). Flies were usually around the AH lure within 2 min of deployment, whereas few flies fed on GF-120 droplets over the 30 min (Table 2). In the additional test on 15 June at the Zillah site, numbers of flies near the GF-120 droplets and the control branch were similar, even in the absence of the AH lure (GF-120, 0.56 ± 0.56 flies/2 min; control, 0.57 ± 0.30 flies/2 min; $P > 0.05$). On a few occasions in tests 1 and 2, the same fly stayed in one location near GF-120 droplets for >10 min, suggesting arrestment caused by the bait, but some flies also remained in the same locations near control branches or near the AH lure for this length of time.

Table 1. Mean ± SE numbers of 3–7-d-old *R. indifferens* landing on the top and bottom of artificial leaves per 2 min treated with three GF-120 concentrations (vol:vol) on top of leaves in the laboratory over 1 h

Concn (%)	*n*	% on leaves[a]	No. on top	No. on bottom	No. feeding
			Starved flies (no food 16–20 h before tests)		
0	5	1.8 ± 1.1a	0.05 ± 0.03a	0.65 ± 0.43a	0.01 ± 0.01a
0.6	5	2.2 ± 0.7a	0.54 ± 0.20ab	0.35 ± 0.15a	0.10 ± 0.03a
4.8	5	3.6 ± 0.8ab	1.14 ± 0.27b	0.21 ± 0.11a	0.22 ± 0.04a
40.0	5	8.1 ± 3.1b	2.73 ± 0.96c	0.51 ± 0.32a	0.64 ± 0.17b
			Nonstarved flies (access to yeast-sugar food immediately before tests)		
0	5	0.0 ± 0.0a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
0.6%	5	1.0 ± 0.6a	0.00 ± 0.00a	0.40 ± 0.24a	0.00 ± 0.00a
4.8%	5	0.7 ± 0.7a	0.06 ± 0.06a	0.21 ± 0.21a	0.00 ± 0.00a
40.0%	5	1.2 ± 0.5a	0.08 ± 0.08a	0.38 ± 0.23a	0.01 ± 0.01a

A volume of 1000 μl was placed on top of four leaves (250 μl per leaf). For each replicate test within a concentration, a plant treated with water only also was present. There were 20 females and 20 males per replicate. Means followed by the same letters within columns are not significantly different (ANOVA, Fisher's LSD test, $P > 0.05$).

[a] On top and bottom of leaves.

Table 2. Numbers ± SE of *R. indifferens* observed per 2 min near a 55.6% GF-120 (vol:vol) solution applied on cherry leaves versus numbers of flies near or on an AH lure in the field in Zillah and Roslyn, WA, 2004

Date	*n*	Control	AH	GF-120	No. feeding
		Zillah, WA–2,500 μl applied on five leaves (500 μl/leaf) within a branch			
20 May	3	1.13 ± 0.26a	6.64 ± 1.28b	0.58 ± 0.28a	3
27 May	3	0.00 ± 0.00a	3.22 ± 1.14a	1.20 ± 0.89a	0
2 June	3	1.80 ± 0.73a	6.20 ± 3.10a	1.43 ± 0.57a	1
9 June	3	0.19 ± 0.19a	1.74 ± 0.39b	0.22 ± 0.22a	0
24 June	3	0.00 ± 0.00a	0.73 ± 0.15b	0.00 ± 0.00a	0
		Roslyn, WA–5,000 μl applied on five leaves (1000 μl/leaf) within a branch			
1 July	4	0.00 ± 0.00a	0.60 ± 0.44a	0.52 ± 0.49a	0
8 July	6	0.04 ± 0.04a	2.18 ± 0.72b	0.87 ± 0.62ab	1
12 July	5	0.00 ± 0.00a	3.09 ± 0.62b	0.25 ± 0.12a	0
26 July	6	0.17 ± 0.11ab	1.49 ± 0.75b	0.00 ± 0.00a	0

AH, 10-ml ammonium hydroxide in Nalgene bottle, 2 mg of ammonia/h release rate. Observations were made for 30 min per tree. Means followed by the same letters within rows are not significantly different (ANOVA, Fisher's LSD test, $P > 0.05$).

Experiment 3: Feeding Responses of Starved Flies to GF-120 Concentrations in Vials. Only 29–65% of both sexes fed on GF-120 droplets, with 4.8% GF-120 eliciting the greatest response (Table 3). Feeding responses between the sexes differed, but mortality of the sexes after ingestion was similar. Males fed for shorter durations ($F = 7.55$; df = 3, 80; $P = 0.0002$) and consumed less of 0.6 than 4.8 and 40.0% GF-120 ($F = 6.14$; df = 3, 80; $P = 0.0008$), whereas females fed on all concentrations for similar durations ($P > 0.05$) and consumed similar amounts of each ($P > 0.05$) (Table 3). Consumption of 0.6% GF-120 by both sexes resulted in 60% mortality. Four males and four females that consumed 0.00012–0.00206 and 0.000003–0.00191 mg of GF-120 in the 0.6% concentration, respectively, were still alive by 14 d after feeding. In addition, one female that consumed 0.00372 mg of GF-120 in the 4.8% concentration was still alive 14 d after feeding, for a total mortality of 95.2% at this concentration. All males and females that consumed 40.0% GF-120 died within 1 d. Some flies that contacted the GF-120 did not feed. It was unclear whether mortality of nonfeeders presented with 4.8 and 40.0% GF-120 was related to contact with spinosad, because mortality of nonfeeders was high (Table 3). Four of six males that contacted but did not feed on these concentrations died; two of eight females did.

Experiment 4: Mortality of Starved and Nonstarved Flies Exposed to GF-120 Concentrations in Cages. Starved and nonstarved flies exposed to increasingly higher GF-120 concentrations suffered greater mortality (Table 4), although even the lowest concentration caused ≥9 times the mortality seen in the control. Mortality was higher in starved than nonstarved flies. Concentrations of ≥16.7% GF-120 resulted in 85.5–99.0% mortality in starved flies, whereas these resulted in only 37.0–81.8% mortality in nonstarved flies over the 4 d. For starved flies, the estimated LC_{50} values of spinosad at days 1, 2, 3, and 4 were 1.5, 1.1, 0.9, and 0.7 ppm, respectively, whereas for nonstarved flies, they were 122.0, 30.2, 19.2, and 13.8 ppm, 20–81 times higher. The respective estimated LC_{90} values for starved flies were 69.0, 30.4, 16.5, and 13.8 ppm and for nonstarved flies they were 23,218.0, 4,868.6, 1,456.4, and 645.6 ppm. Two-way ANOVA indicated there were significant effects of starvation category ($F = 98.50$; df = 1, 100; $P < 0.0001$) and concentration ($F = 33.05$; df = 10, 100; $P < 0.0001$) on mortality at day 4, but there was also a significant interaction between the two factors ($F = 1.91$; df = 10, 100; $P = 0.0523$). Starved flies reached high plateau mortalities at low concentrations, whereas nonstarved flies reached high mortalities only at higher concentrations (Table 4).

Table 3. Mean ± SE feeding durations (seconds) and mean amounts consumed (milligrams) of three GF-120 concentrations (vol:vol) by individual 4–6-d-old male and female *R. indifferens* inside vials

Concn (%)	(AI) ppm	*n*	% fed	Feeding duration	Amount	% mortality by 14 d	
						Fed	Not fed
Males							
0	0	11	9.1	0.6 ± 0.6a	0.0138 ± 0.0138a	0.0	25.0
0.6	1	34	29.4	6.2 ± 2.5a	0.0722 ± 0.0307a	60.0	16.7
4.8	11	18	55.6	49.8 ± 14.0b	0.4894 ± 0.1483b	100.0	75.0
40.0	89	21	52.4	62.1 ± 18.0b	0.3280 ± 0.0999b	100.0	60.0
Females							
0	0	11	9.1	1.1 ± 1.1a	0.0337 ± 0.0337a	0.0	11.1
0.6	1	16	62.5	30.8 ± 12.8a	0.3183 ± 0.1298a	60.0	16.7
4.8	11	17	64.7	20.0 ± 7.8a	0.3615 ± 0.0999a	90.9	33.3
40.0	89	21	52.4	26.0 ± 7.6a	0.2318 ± 0.0702a	100.0	22.2

Means followed by the same letters within columns are not significantly different (ANOVA, Fisher's LSD test, $P > 0.05$).
Flies had access to yeast-sugar food before starvation; flies starved 16–20 h prior to tests.

Table 4. Mean ± SE cumulative percentage of mortality of 3–7-d-old starved and nonstarved *R. indifferens* flies exposed to 10 GF-120 concentrations (vol:vol) in dishes for 1 h at days 1–4 postexposure

Concn (%)	(AI) ppm	Day 1		Day 2		Day 3		Day 4	
		Starved	Nonstarved	Starved	Nonstarved	Starved	Nonstarved	Starved	Nonstarved
0	0	4.2 ± 2.0	1.5 ± 0.8	5.0 ± 2.2	2.0 ± 0.8	5.0 ± 2.2	2.0 ± 0.8	7.1 ± 2.7	2.0 ± 0.8
0.6	1	41.2 ± 9.9	14.0 ± 2.9	49.4 ± 8.5	22.0 ± 3.4	**57.4 ± 10.3**	22.0 ± 3.4	**61.8 ± 11.3**	22.0 ± 3.4
1.2	3	**66.7 ± 14.5**	23.0 ± 5.6	**67.7 ± 14.8**	30.0 ± 6.5	68.8 ± 15.1	30.0 ± 6.5	70.9 ± 15.6	30.0 ± 6.5
2.4	6	71.0 ± 10.6	20.0 ± 6.9	76.4 ± 10.7	33.0 ± 9.4	81.5 ± 11.1	34.0 ± 9.3	86.9 ± 11.8	40.0 ± 12.5
4.8	11	73.4 ± 11.8	23.0 ± 6.0	85.7 ± 9.4	36.0 ± 10.9	85.7 ± 9.4	42.0 ± 11.9	88.8 ± 7.5	44.0 ± 12.1
9.1	21	85.5 ± 9.2	32.0 ± 5.6	87.6 ± 8.3	47.1 ± 7.9	89.7 ± 7.6	48.2 ± 8.2	89.7 ± 7.6	**51.2 ± 9.3**
16.7	39	88.0 ± 5.4	43.7 ± 11.1	92.0 ± 5.1	**61.2 ± 9.8**	95.2 ± 3.6	**69.8 ± 9.9**	95.2 ± 3.6	72.9 ± 9.0
20.0	46	85.5 ± 6.8	**54.7 ± 9.6**	89.8 ± 6.7	65.8 ± 9.3	96.6 ± 2.1	77.8 ± 8.7	96.6 ± 2.1	81.8 ± 6.4
25.0	57	86.7 ± 6.5	40.0 ± 13.0	96.5 ± 1.8	55.0 ± 10.7	98.6 ± 1.4	62.0 ± 10.6	98.6 ± 1.4	73.0 ± 11.6
33.3	75	91.7 ± 4.6	37.0 ± 10.1	94.0 ± 4.8	53.0 ± 12.9	99.0 ± 1.0	54.0 ± 13.1	99.0 ± 1.0	58.0 ± 13.3
40.0	89	91.8 ± 2.6	50.4 ± 7.6	95.5 ± 2.0	58.4 ± 7.3	98.0 ± 2.0	64.4 ± 8.0	99.0 ± 1.0	70.5 ± 7.2

Starved, no food 16–20 h before testing; nonstarved, food up to testing. $n = 5$, except for 0 concentration ($n = 12$, starved; $N = 10$, nonstarved). Twenty flies (10 males and 10 females) were used per replicate. In bold, first value within column to exceed 50%.

Experiment 5: Mortality and Oviposition of Flies Exposed to GF-120 Concentrations on Cherries in Cages. In test 1 (Table 5), mortality of flies exposed to treated cherries was greater at each higher GF-120 concentration at days 1 and 2 ($F = 83.99$ and 220.07; df = 3, 24; $P < 0.0001$). Despite this, flies oviposited in all treated cherries, with egg numbers highest in the 0.6% and lowest in the 40.0% GF-120 treatment ($F = 7.34$; df = 3, 8; $P = 0.0110$). The number of larvae from the 0.6% GF-120 treatment was similar to that from the control, but no larvae emerged from the 4.8 and 40.0% GF-120 treatments ($F = 3.82$; df = 3, 12; $P = 0.0394$) (Table 5).

In test 2, the pattern of mortality was similar to that in test 1 (days 1–4: $F = 15.11$–36.42; df = 3, 11; $P = 0.0003$ to <0.0001) (Table 5). Eggs were obtained from only one or two replicates of the control and each treatment because most cherries molded by day 4, making eggs difficult to find. Numbers of eggs/fruit/female in the control, 0.6, 4.8, and 40.0% GF-120 were 5.9 ± 2.7, 10.0 ± 4.7, 18.0 (one replicate), and 0.0 ± 0.0, respectively. This indicated that, as in test 1, the spinosad in bait did not kill gravid flies quickly enough to prevent oviposition.

Experiment 6: Field Spray Tests of GF-120 Concentrations. In the field (Table 6), there were no significant differences in numbers of trapped flies in control and GF-120-treated trees in Yakima or Moxee ($P > 0.05$). However, larval infestations were greater in control than treated trees at both sites (Yakima: $F = 8.14$; df = 3, 15; $P = 0.0019$; Moxee: $F = 8.05$; df = 3, 9; $P = 0.0065$). Larval infestations in GF-120 treatments in Yakima and Moxee were 79–94 and 88–91% lower than in controls, respectively (Table 6). Although no significant differences were seen among the three GF-120 concentrations, there were numerical decreases in infestations with increasing concentrations at both sites (Table 6). Fruit from one of three trees treated with 0.6% GF-120 in Yakima did not have an infestation.

Discussion

Our laboratory results indicate that a concentration of 40.0% GF-120 was more attractive to *R. indifferens* than the 0.6% concentration, but that under the test conditions, the attraction was weak and flies responded to it only if they were starved of sugar and

Table 5. Mean ± SE cumulative percentage of mortality of ≥14-d-old *R. indifferens* at 1 and 2 d after exposure to cherries treated with three GF-120 concentrations (vol:vol) and mean ± SE eggs/female/three fruit; 2- and 4-d exposures

Concn (%)	(AI) ppm	*n*	Day 1	Day 2	Eggs/♀/3 fruit[a]	No. larvae/3 fruit[b]
Test 1 (2-d exposure)						
0	0	7	0.0 ± 0.0a	1.4 ± 1.4a	15.7 ± 5.9a	1.2 ± 0.8ab
0.6	1	7	23.6 ± 7.0b	54.3 ± 4.6b	9.4 ± 1.6ab	2.2 ± 0.8a
4.8	11	7	68.9 ± 3.7c	95.5 ± 2.1c	3.3 ± 1.4bc	0.0 ± 0.0b
40.0	89	7	88.9 ± 4.2d	100.0 ± 0.0d	0.5 ± 0.1c	0.0 ± 0.0b
Test 2 (4-d exposure)			Day 1	Day 2	Day 3	Day 4[c]
0	0	4	2.5 ± 2.5a	5.0 ± 2.9a	7.5 ± 4.8a	7.5 ± 4.8a
0.6	1	4	7.5 ± 4.8ab	12.5 ± 4.8ab	22.5 ± 4.8ab	40.0 ± 13.5b
4.8	11	3	26.7 ± 3.3b	46.7 ± 3.3c	50.0 ± 0.0b	86.7 ± 3.3c
40.0	89	4	75.0 ± 15.0c	90.0 ± 7.1d	90.0 ± 7.1c	100.0±0.0d

Tests 1 and 2: 10 flies (five males and five females) per replicate. Means followed by the same letter within columns are not significantly different (ANOVA, Fisher's LSD test, $P > 0.05$).

[a] From three of seven replicates.

[b] From four of the seven replicates.

[c] Eggs recovered in all treatments except 40.0% GF-120; cherries molded and complete counts not made.

Table 6. Effects of three GF-120 concentrations (vol:vol) on mean ± SE numbers of adult *R. indifferens* caught per sticky yellow trap on single cherry trees and mean ± SE numbers of larvae per fruit at two sites in Washington, May to June 2004

Concn (%)	Yakima			Moxee		
	n	No. flies[a]	No. larvae/fruit	*n*	No. flies[a]	No. larvae/fruit
0	9	73.0 ± 15.1a	0.743 ± 0.141a	4	14.2 ± 2.1a	0.007 ± 0.003a
0.6	3	55.0 ± 36.5a	0.158 ± 0.083b	4	11.8 ± 3.4a	0.001 ± 0.001b
4.8	3	15.7 ± 12.7a	0.053 ± 0.012b	4	9.8 ± 4.4a	0.001 ± 0.001b
40.0	4	20.5 ± 14.7a	0.045 ± 0.021b	4	6.2 ± 1.3a	0.0006 ± 0.0006b

Spray volume of 225 ml per tree. Yakima: five applications; 200 cherries per tree; Moxee: four applications; 231–328 cherries per tree. Means followed by the same letter within columns are not significantly different (ANOVA, Fisher's LSD test, $P > 0.05$).

[a] Yakima: totals over 35 d; Moxee: totals over 17 d.

protein for 16–20 h. It is possible some flies landed on the leaves by accident and then stayed on the leaves because of the odor of the droplets. The 8% overall response of starved *R. indifferens* to 40.0% GF-120 is similar to the 14% response of protein-starved *R. mendax* to 100% Solbait (Barry and Polavarapu 2004) and to the 10% response of the melon fly, *Bactrocera cucurbitae* Coquillett, to 40% GF-120 (Revis et al. 2004). Ammonia may have been one of the attractants emitted from GF-120. However, because of the low percentage of ammonia in GF-120, the amounts emitted were probably too low to attract many flies. Ammonia also may not have been the only attractant from GF-120. *Ceratitis capitata* is attracted to fresh Nulure bait (Prokopy et al. 1992), but ammonia was not among the 43 compounds isolated from it (Buttery et al. 1983). Also, *A. ludens* is highly attracted to Mazoferm, but ammonia was not one of the 19 compounds detected in this bait (Lee et al. 1997). It is possible, though, that the chemical analyses conducted could not detect ammonia from Nulure and Mazoferm. Nonstarved *R. indifferens* did not respond to GF-120, suggesting that attractants more powerful than those from fresh GF-120 are needed to stimulate well-fed flies. Whether flies in nature behave like starved or nonstarved flies has not been studied, but flies starved for 16–20 h contain similar amounts of sugars as wild flies (W.L.Y., unpublished). Five- to 7-d-old *R. indifferens* deprived of protein but not sucrose also were not attracted to GF-120, although *C. capitata* deprived of protein for 8 d from eclosion with continuous access to sucrose were more responsive to fresh Nulure bait than those deprived for only 0–3 d (Prokopy et al. 1992). The low responses of *R. indifferens* to GF-120 do not preclude the possibility that although flies are not attracted to the bait from far distances within trees, they are attracted to it at very close range (i.e., within a leaf). In addition, aging GF-120 droplets will likely change the volatile profiles and can apparently increase or decrease attractiveness. In *A. ludens*, there was no response to GF-120 until 16 h after release (Moreno and Mangan 2003). However, in *B. cucurbitae*, GF-120 aged for 2 h was less attractive than fresh bait (Revis et al. 2004). The effect of mating status and age on responsiveness were not studied, but we chose to use young flies for tests because baits need to kill flies before they develop eggs to prevent oviposition.

Consistent with our laboratory results, *R. indifferens* rarely landed near fresh 55.6% GF-120 during 30-min observations in field cherry trees, indicating wild flies were not strongly responsive to the fresh bait. Nulure bait also was ineffective in attracting the apple maggot, *Rhagoletis pomonella* (Walsh), in the field (Hendrichs et al. 1990). In *R. indifferens*, it was possible the 55.6% GF-120 was slightly repellent due to a high ammonium acetate level. High levels of ammonium acetate repel *A. ludens* from McPhail traps (Moreno and Mangan 2003), and in *R. mendax*, 1 and 4% ammonium acetate seemed slightly repellent compared with 0.25% ammonium acetate (Barry and Polavarapu 2004). Possibly the acetate component (which produces the vinegar odor), not the ammonia, repelled the flies. Our observations suggest *R. indifferens* were not arrested near the GF-120 more than near the lures containing 10 ml of ammonium hydroxide (which almost certainly emitted more ammonia) or control branches in the field. However, Solbait seemed to arrest *R. mendax* more than other baits in the laboratory (Barry and Polavarapu 2004). GF-120 also seemed to arrest *R. mendax* in the field (Pelz et al. 2005).

Only moderate numbers of starved *R. indifferens* fed on GF-120 droplets inside small vials, perhaps because some flies were not attracted or were even repelled by the odor inside the vials. The responsive flies either were attracted by the odor or encountered the bait by random movement, whereupon they were stimulated to feed by protein, sugar, or both. Males fed longer on and consumed more of 4.8 and 40.0% than other GF-120 concentrations, whereas females fed for similar times and consumed similar amounts of all concentrations. Females responded equally to all concentrations perhaps because their need for protein is greater than that of males, e.g., as in *R. pomonella* (Webster et al. 1979). Several factors may affect feeding responses. In *R. indifferens*, 2% sucrose did not stimulate as much feeding as 20% sucrose (Yee 2003), but females in the current study responded to GF-120 with only 0.2–11.9% sugar (0.6–40.0% GF-120), so perhaps low amounts of protein helped stimulate feeding. *R. mendax* fed more on 100% Solbait with only 2% protein than on AY50% bait with 25% protein (Barry and Polavarapu 2004). The bait's pH also may affect amounts consumed, because a pH of 6.4 is most stimulatory for *R. pomonella* (Hu et al. 1999), perhaps

contributing to the feeding on 0.6% GF-120 (also with a pH of 6.4) by female *R. indifferens.*

In the feeding response experiment, ingested spinosad in GF-120 proved highly toxic to male and female *R. indifferens.* Based on survival of flies that consumed 0.6% GF-120, males and females could ingest 0.0002–0.0001μg spinosad/fly and survive, but ingestion of only $\approx$0.0005–0.006 μg spinosad/fly was lethal. The results suggest the toxicity of ingested spinosad is nearly as high as that of the contact organophosphate insecticide dimethoate (Zwick et al. 1975), although direct comparisons need to be made.

Starved *R. indifferens* in cages with $\geq$16.7% GF-120 suffered maximal mortality, although mortality above that of the controls was seen with only 0.6% GF-120. This and previous findings suggest high GF-120 concentrations and food deprivation are two vital factors that contribute to increased fly mortality, even when flies have access to protein before starvation. Mortality resulted from ingesting the bait, but flies that did not feed may also have contacted the spinosad and died. Flies may have ingested some spinosad during preening after contacting it with their tarsi. In *C. capitata,* either ingestion of or residual contact with spinosad caused high mortality (Adán et al. 1996). Estimates of LC_{50} values of flies exposed to GF-120 were consistent with measured mortality of flies from the feeding experiment, but these estimates no doubt would have been higher had all the flies fed on the solutions. Results with *R. indifferens* are in agreement with those for other fruit flies. In *R. pomonella,* a 48-h exposure to only 3.2 ppm spinosad on apples caused 65% mortality, whereas 10–316 ppm caused >90% mortality. Addition of 1–10% sugar to 1 ppm spinosad resulted in $\approx$38–75% mortality (Reissig 2003). In *C. capitata,* LC_{50} values of spinosad when ingested were 3.49 and 0.18 ppm at 14 h and 7 d after treatment (Adán et al. 1996), and 90% mortality was seen in females that ingested 4.2 ppm (Nestel et al. 2004). The increases in mortality of starved and nonstarved *R. indifferens* exposed to low concentrations over 4 d suggest ingestion of or contact with small quantities of GF-120 can result in a slow lethal effect.

Mortality of gravid *R. indifferens* exposed to cherries treated with higher GF-120 concentrations was greater than with the lower concentrations, but even the highest concentration could not prevent oviposition. The results clearly indicate GF-120 needs to be applied before flies are first able to lay eggs (at 5–10 d old; Frick et al. 1954) or the material will be ineffective, which also is the case with *R. pomonella* exposed to insecticides on apples (Reissig 2003). Either the insecticide or bait component can have a significant effect on oviposition. In *R. pomonella,* 25 and 100% Nulure bait on apples totally suppressed oviposition (Mohammad and AliNiazee 1991) and 316 ppm of spinosad without bait on apples resulted in 98% reduction in oviposition (Reissig 2003). *R. indifferens* larvae developed in cherries treated with 0.6% GF-120, suggesting low amounts of spinosad are ineffective in killing the eggs or larvae in fruit. Whether 4.8% and 40.0% GF-120 affected these stages remains unanswered, although spinosad is known to have translaminar activity in leaf tissue (Dow AgroSciences 2004). The possibility that lethal concentrations are absorbed by eggs after ingestion of the bait needs to be examined.

The results of the field spray tests were not entirely consistent with the observed GF-120 concentration effects in the laboratory, but they showed that GF-120 was effective in greatly suppressing larval infestations in cherries. There are several possible explanations for the lack of a concentration effect on infestations, including variations in initial fly numbers among trees, rain that may have diluted the spray deposits (after three of five sprays in Yakima), the short residual activity of spinosad, and immigration of gravid flies from adjacent and nontreated trees (in Moxee). Spray results were consistent with those obtained for the walnut husk fly, *Rhagoletis completa* Cresson, in walnuts, where larval infestations were reduced 75–87%, and where no GF-120 rate effect was detected (Van Steenwyk et al. 2003), and for *R. mendax* in blueberries, where infestations were reduced 85 and 98% (Pelz et al. 2005). In contrast, GF-120 sprays were ineffective against *R. pomonella* in apples (Reissig 2003) or reduced infestation in apples by only 67% (Pelz et al. 2005).

In conclusion, evidence from this study indicates that fresh 40.0% GF-120 was attractive to *R. indifferens* in the laboratory, but that flies were not attracted to fresh GF-120 from far distances within trees, suggesting attraction to the bait was not solely responsible for the suppression of infestations in spray tests. Based on this evidence, a reasonable hypothesis is that suppression was caused in large part by flies finding the bait through normal movement over large areas over a 6–10-d period (the spray interval in this study). GF-120 probably is attractive at close range, and after contacting the bait, the flies were stimulated to feed on the sugar and protein. After the bait was ingested, the high toxicity of spinosad ensured most if not all flies died. Perhaps due in part to the low attractiveness of flies to GF-120, not all the flies found the bait droplets early enough to prevent oviposition and subsequent infestations. Although the effects of aged GF-120 on fly responses still need to be determined, the results suggest the efficacy of GF-120 needs to be improved by reformulating the bait with more attractive components.

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